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A Ritsby Consultants Company

Standard Operating Procedure

Zooplankton Sampling

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1 COMMUNITY COMPOSITION

1.1 Sample Collection

Samples for analysis of zooplankton community composition are to be collected using a 19 cm (or alternate) diameter, fine mesh (i.e., 60 µm) plankton net, vertically hauled through the top X m of the water column (consult the study design to determine the required sampling depth). Zooplankton is collected as follows:

a. Lower the plankton net to the required depth, and then slowly draw the net back up to the surface. Use a hand over hand technique, with a steady, unhurried motion at a rate of 0.5 m/s.

b. Once the net is at the surface, rinse the outside of the net from the top downward using ambient water to wash adhered zooplankton into the cod-end (i.e., removable collection cup).

c. Rinse the net a second time to ensure all zooplankton have been transferred to the collection cup.

d. Disconnect the removable cup containing the sample, and decant into a pre-labelled plastic sample jar.

e. Rinse the cup with deionized water, and pour the rinsate into the sample bottle to ensure all zooplankton are collected.

f. Repeat the above procedure from steps a) to e), as required based on the study design (i.e., a composite of X hauls) to form a single community sample at each sampling location.

g. Preserve the sample in a 10% buffered formalin solution[1] (i.e., powdered borax and formaldehyde) equivalent to 10% of the sample volume.

    - Formalin is a carcinogen and an irritant to workers, so protective gloves, fit tested respiratory gear, and eye protection are required.
    - Preserve the sample as soon as practical after sampling. After adding the preservative, gently mix the sample several times to ensure that the preservative has thoroughly penetrated the sample. Samples can be kept at room temperature following preservation.

[1] Preservation directly in ethanol is sometimes less effective, resulting in damage to organisms that impairs taxonomic identification.

- Ship samples preserved in formalin to the analytical laboratory within 3 weeks of collection to prevent acidic degradation of organisms by the formalin (i.e., the taxonomy lab will remove the formalin preservative and replace it with 70% ethanol to avoid decalcification of organisms).
- Always ship samples by ground transportation (not air).

2 TISSUE CHEMISTRY

2.1 Sample Collection

Zooplankton samples for tissue chemistry should be collected using a mesh plankton net (e.g., 80 µm mesh, 30 cm diameter), vertically hauled through the top X m of the water column (consult the study design to determine the appropriate sampling depth).

a. Lower the plankton net to the required depth, and then slowly draw the net back up to the surface. Use a hand over hand technique, with a steady, unhurried motion at a rate of 0.5 m/s.

b. Once the net is at the surface, rinse the outside of the net from the top downward using ambient water to wash adhered zooplankton into the cod-end (i.e., removable collection cup).

c. Rinse the net a second time to ensure all zooplankton have been transferred to the collection cup.

d. Disconnect the removable cup containing the sample, and decant into a plastic tub.

e. Rinse the cup with deionized water, and pour the rinsate into the sample bottle to ensure all zooplankton are collected.

f. Repeat the above procedure from steps a) to e), as required based on the study design (i.e., a composite of X hauls) to form a single tissue sample at each sampling location. Sampling will typically require more than one vertical haul to obtain sufficient weight for analysis.

g. Following collection of the final haul, pour the contents of the plastic tub into the plankton net to consolidate the sample and allow for removal of as much water as possible. Use the mesh on the side of the plankton net to remove as much water from the sample/collection cup as possible.

h. Remove the collection cup from the net and transfer the contents into a sterile cryovial. Transfer the samples to a cooler with ice until they can be frozen later in the day.

i. At the end of the field program, ship samples by ground transportation (not air), ensuring that there is sufficient ice in the cooler that samples reach the laboratory frozen.

3 FROM THE 2018 TO 2020 KOOCANUSA STUDY DESIGN

3.1 General Information

Zooplankton community samples will be collected in 2018 at five stations both upstream and downstream of the Elk River[2]. Zooplankton tissue will be collected annually (2018 to 2020) from the same stations as community samples.

3.2 Sample Collection

3.2.1 Community Composition

Samples for analysis of zooplankton community composition will be collected using a 19 cm diameter, fine mesh (i.e., 60 µm) plankton net, vertically hauled through the entire water column at each sampling station, using methods described in Section 1. The plankton net will be lowered to a depth of 1.5 m from the sediment-water interface (to avoid disturbing the sediment, potentially resulting in addition of benthic organisms to the sample). A total of three vertical hauls will be collected and composited to form a single community sample at each of the five sampling stations upstream and downstream of the Elk River. Each sample will be transferred into a pre-labelled plastic sampling jar, and preserved to a level of 10% buffered formalin. Samples will be maintained at room temperature until shipment to a qualified laboratory for taxonomic identification.

3.2.2 Tissue Chemistry

Zooplankton will be collected for analysis of tissue chemistry using an 80 µm mesh net (30 cm diameter) vertically hauled through the entire water column, and vertically hauled through the top 10 m of the water column (i.e., two separate samples). A slightly larger mesh size will be used for tissue collection (compared to community samples) so that the sample mostly consists of zooplankton and is not confounded by the presence of phytoplankton. A total of 10 vertical hauls will be collected at each of the five stations upstream and downstream of the Elk River for samples collected through the entire water column, and samples collected through the top 10 m. Hauls will be composited and filtered through the net a second time to remove as much water as possible, and to allow for sufficient sample weight for analysis. Samples will then be transferred to sterile cryovials and frozen, pending shipment to a qualified laboratory.

[2] The Ministry of Environment and Climate Change Strategy (ENV) indicated in a letter on June 8th, 2018 that Teck must add June zooplankton community and tissue sampling to assess seasonal changes for all years covered in the sampling design.

3.3 Laboratory Analysis

3.3.1 Community

Zooplankton samples, after standing for 72 hours, will be decanted (60 µm filter on vacuum hose, back flushed) to 45 mL glass vials to standardize volume (40 mL) for analyses and long term storage. Samples will be analyzed for species composition, abundance, and biomass of crustaceans and rotifers. Each sample will undergo the following three levels of analysis:

- 1/10, 1/20, 1/40 or 1/80 (depending on amount of zooplankton in sample) of each sample will be examined under a compound microscope at 63× to 160×, and a minimum of 200 organisms will be identified to species (crustaceans) or lowest possible level (rotifers), and assigned to instar size categories. Additionally, lengths (± 15 µm) of female and male adult specimens (n=20) of dominant species will be measured in representative samples for biomass determinations;
- a sub-sample, representing 10 to 20% of the sample volume, will be examined under a stereoscope at 12× magnification for mature and gravid individuals of larger species, and for individuals of less abundant species. They will be identified, enumerated, and assigned to size classes; and
- the entire sample will be examined under stereoscope to improve abundance/biomass estimates for the largest, less numerous species.

Under a compound microscope, Cyclopoida and Calanoida specimens (mature and immature) will be identified to the species level, with the exception of nauplii (N1-N6) which will be classified as either Calanoida (small or large) or Cyclopoida (small or large). Cladocera will be identified to the species level, while rotifers will be identified to genus. Zooplankton abundance will be reported as individuals per litre (ind/L) based on volumes calculated from net mouth area and sample haul depth. Taxonomic identifications are based primarily on Brooks[3] (1957), Wilson[4] (1959), and Yeatman[5] (1959).

Biomass estimates for each species will be determined from:

[3] Brooks, J. L. 1957. The systematics of North American Daphnia. Mem. Conn. Acad. Arts Sci. 13: 1-80.

[4] Wilson, M.S. 1959. Calanoida, p. 738-794. In W.T. Edmondson [ed.] Freshwater Biology. 2nd ed. John Wiley & Sons, New York, NY.

[5] Yeatman, H. C. 1959. Cyclopoida, p. 795-815. In W.T. Edmondson [ed.] Freshwater Biology. 2nd ed. John Wiley & Sons, New York, NY.

- abundances of adults multiplied by mean adult wet weights developed from measured lengths (n=20 per adults of dominant species in representative samples), and length-weight relationships from Malley et al.[6] (1989); and
- abundances of various immature instar categories multiplied by weights of respective size categories determined from length-weight regressions (Malley et al. 1989).

Additional size measurements are made on less common specimens for biomass calculations. Zooplankton biomass is reported in micrograms (wet weight) per litre (µg/L). Digital microscopic images of selected specimens are provided with the analytical data.

Sub-sampling accuracy will be assessed by performing replicate counts on 10% of samples. Replicate samples will be chosen at random and processed at different times from the original sample to reduce bias.

3.3.2 Tissue Chemistry

Zooplankton samples will be shipped to a qualified laboratory for analysis of metals (including mercury) and selenium using high-resolution inductively coupled plasma mass spectrometry (HR-ICP-MS). The laboratory will freeze dry the samples prior to analysis. Concentrations will be reported on a dry weight basis. Accuracy and precision of data will be judged based on ability to achieve minimum laboratory reporting limits, replicate analysis of a minimum of 10% of samples, as well as a comparison to certified reference materials.

[6] Malley, D.F., S.G. Lawrence, M.A. MacIver, and W.J. Findlay. 1989. Range of variation in estimates of dry weight for planktonic Crustacea and Rotifera from temperate North American lakes. Can. Tech. Rep. Fish. Aquat. Sci. 1666: iv+49p.